Since January 2020 Elsevier has created a COVID-19 resource centre with free information in English and Mandarin on the novel coronavirus COVID-19. The COVID-19 resource centre is hosted on Elsevier Connect, the company's public news and information website.

Editorial

JOURNAL OF HEPATOLOGY

Unraveling the role of liver sinusoidal endothelial cells in COVID-19 liver injury

Antonio Saviano[1,2,3,*], Thomas F. Baumert[1,2,3,4,*]

[1]*Institut de Recherche sur les Maladies Virales et Hépatiques (IVH), Inserm U1110, Strasbourg, France;* [2]*Institut Hospitalo-Universitaire, Pôle Hépato-digestif, Nouvel Hôpital Civil, Hôpitaux Universitaires de Strasbourg, Strasbourg, France;* [3]*University of Strasbourg, Strasbourg, France;* [4]*Institut Universitaire de France (IUF), Paris, France*

See Article, pages 647–658

Liver injury is common during SARS-CoV-2 infection. Abnormalities in liver tests can be found in up to 50% of infected patients.[1] In most cases, COVID-19 liver test perturbations comprise liver aminotransferase elevation and correlate with disease severity (defined as intensive care unit admission and mortality).[2,3] Liver injury is generally mild and does not lead to liver failure in patients without advanced liver disease. Patients with decompensated cirrhosis, however, are at a significant risk of developing acute-on-chronic liver failure.[4] Although several mechanisms for these observations have been postulated, the pathogenesis of liver injury in COVID-19 is largely unknown. Liver cells such as hepatocytes seem to be poorly permissive to SARS-CoV-2 infection. ACE2 and TMPRSS2, the 2 major viral cell entry factors, are expressed at only low levels in hepatocytes and non-parenchymal cells.[3,5] Even though SARS-CoV2 RNA has been detected in the livers of COVID-19 patients,[6] functional studies demonstrating robust SARS-CoV2 infection of liver cells are pending.[3]

Histopathological studies of COVID-19 livers reported an increased prevalence of moderate steatosis, mild lobular and portal inflammation, and sinusoidal thrombosis.[3] Moreover, COVID-19 has been reported to be more severe in patients with obesity and metabolic syndrome.[3,7,8] Whereas steatosis, lobular and portal inflammation are common features of metabolic liver disease, sinusoidal thrombosis is a potential candidate for a specific feature of COVID-19-related liver injury. Indeed, COVID-19 is a prothrombotic disease associated with a high risk of venous thrombosis, pulmonary embolism and endotheliopathy,[9] and sinusoidal thrombosis has also been observed in liver histopathological studies of severe COVID-19.[3,10,11]

COVID-19 is characterized by an intense systemic proinflammatory response which can turn into an uncontrolled massive cytokine release able to induce multi-organ failure.[12] Interleukin (IL)-6 has been postulated as a key proinflammatory cytokine in COVID-19 pathogenesis and IL-6 inhibition may improve outcomes and survival of patients with severe disease.[13] In this issue of *Journal of Hepatology*, McConnell, Kawaguchi *et al.*[14] investigated the role of IL-6 signaling in the induction of a pro-coagulatory and proinflammatory phenotype in liver sinusoidal endothelial cells (LSECs), also called endotheliopathy by some authors.[15]

IL-6 is a cytokine produced by macrophages, endothelial cells, T cells and fibroblasts upon stimulation of Toll-like receptor 4, IL-1 or tumor necrosis factor-α.[16] IL-6 signaling involves classical cis-signaling as well as trans-signaling pathways. In the classical pathway, IL-6 signaling is initiated upon association of IL-6 with the membrane-bound IL-6 receptor (IL6-R) which subsequently forms a complex with glycoprotein 130 (gp130). This classical pathway of signal transduction restricts IL-6 signaling to cells expressing IL6-R in the liver, such as hepatocytes, cholangiocytes, Kupffer cells and hepatic stellate cells. IL-6R can also be cleaved at the cell surface by metalloproteinases into a soluble receptor (sIL-6R) which can form a complex with IL-6 and bind to gp130 on the cell surface of IL-6R-negative cells, initiating intracellular IL-6 signaling. Once initiated, the classical and trans-signaling of IL-6 both lead to the activation of the tyrosine kinase JAK1, MAP kinase and STAT1 and STAT3 pathways.

High levels of IL-6 and sIL-6R have been observed in COVID-19 patients but the effect on the liver is still poorly understood.[17] Previous studies have shown that IL-6 not only promotes the acute phase response but also liver regeneration, tumorigenesis and modulation of glucose metabolism.[16]

Aiming to understand the pathogenesis of COVID-19-related liver injury, McConnell, Kawaguchi *et al.*[14] first showed that, in COVID-19 patients, the most common liver pathological features were liver congestion (98%), steatosis (47%), sinusoidal erythrocyte aggregation (44%) and neutrophil infiltration (≥2x that in control livers). Among COVID-19 patients, patients with higher alanine aminotransferase (ALT ≥3x) had higher plasma levels of IL-6 and procoagulation factors, and more importantly, the liver histopathological analysis showed significantly higher intralobular neutrophil infiltration (2x that of patients with lower ALT) as well as trends toward a higher prevalence of steatosis and sinusoidal erythrocyte aggregation. Immunostaining analyses revealed that LSECs in COVID-19 patients were highly

Keywords: Liver sinusoidal endothelial cells; COVID-19; SARS-CoV-2; interleukin-6; liver injury.
Received 1 June 2021; received in revised form 13 July 2021; accepted 13 July 2021; available online 15 July 2021
DOI of original article: https://doi.org/10.1016/j.jhep.2021.04.050.
* Corresponding authors. Address: Inserm U1110, Université de Strasbourg, 3 Rue Koeberlé, F-67000 Strasbourg, France.
E-mail addresses: saviano@unistra.fr (A. Saviano), thomas.baumert@unistra.fr (T.F. Baumert).
https://doi.org/10.1016/j.jhep.2021.07.008

positive for von Willebrand factor (vWF) and showed platelet aggregates at their surface. Moreover, vWF expression and platelet aggregates in LSECs were higher in patients with ALT ≥3x and correlated with intralobular neutrophil infiltration and plasma IL-6 levels. Functional studies in primary LESCs revealed that IL-6 trans-signaling increases the expression of procoagulant factors (Factor VIII and vWF), proinflammatory molecules (IL-6 itself, CXCL1 and CXCL2) and cell adhesion molecules such as ICAM1, P- and E-selectin which are known to mediate and enhance platelet attachment and neutrophil recruitment.[18] Furthermore, their study provides evidence that activated LSECs contribute to increase the systemic inflammatory response by cross talking with hepatocytes and increasing their production of acute phase reactants such as fibrinogen. These perturbations were IL-6 dependent and could be pharmacologically restored by JAK1 inhibitors which are currently under evaluation for the treatment of severe COVID-19.[19] In summary, according to their findings, LSECs respond to IL-6 by acquiring a procoagulant and proinflammatory phenotype which triggers platelet aggregation in sinusoids and liver neutrophil recruitment, contributing to COVID-19-related liver injury (summarized in Fig. 1). Nevertheless, liver neutrophil infiltration appears to be mild (around 2 neutrophils per high power field).

These observations provide a novel concept for the understanding of liver injury in COVID-19 by unraveling a previously undiscovered role of LSECs. The data suggest that COVID-19-related liver changes are likely independent from liver cell infection and that the liver may have a role in sustaining the general proinflammatory response. Even though LSECs do not express IL-6R, McConnell, Kawaguchi *et al.* showed that LSECs respond to IL-6 trans-signaling and are able to cross talk with hepatocytes to produce pro-coagulant and pro-inflammatory molecules as part of the systemic response to the virus.

Collectively, the paper by McConnell, Kawaguchi *et al.* adds another piece to the puzzle in our understanding of the pathogenesis of liver injury in COVID-19. The novel aspects of the McConnell study include the proposed liver-specific findings related to the systemic inflammatory response to SARS-CoV-2, which do not necessarily require direct hepatic parenchymal or non-parenchymal cell infection as the initiating event. An additional strength was the *in vitro* studies demonstrating the beneficial effect of JAK inhibition.

However, some limitations will require further investigation: it remains to be determined whether the endothelial activation described in this paper is COVID-19 specific, because no control patients with another viral infection or sepsis were included. Also, the data cannot infer whether LSEC activation is the only trigger of liver injury because of a lack of detailed mechanistic studies and experimental models. It is noteworthy that COVID-19 thrombosis and severe illness are more frequent in patients with metabolic syndrome and non-alcoholic fatty liver disease (NAFLD).[7,8] In the cohort of patients from the study by McConnell, Kawaguchi *et al.*,[14] there was a high prevalence of steatosis, and it is also possible that, in the context of systemic inflammation, liver steatosis rather than endotheliopathy, or the combination of the 2 elements, may favor liver neutrophil

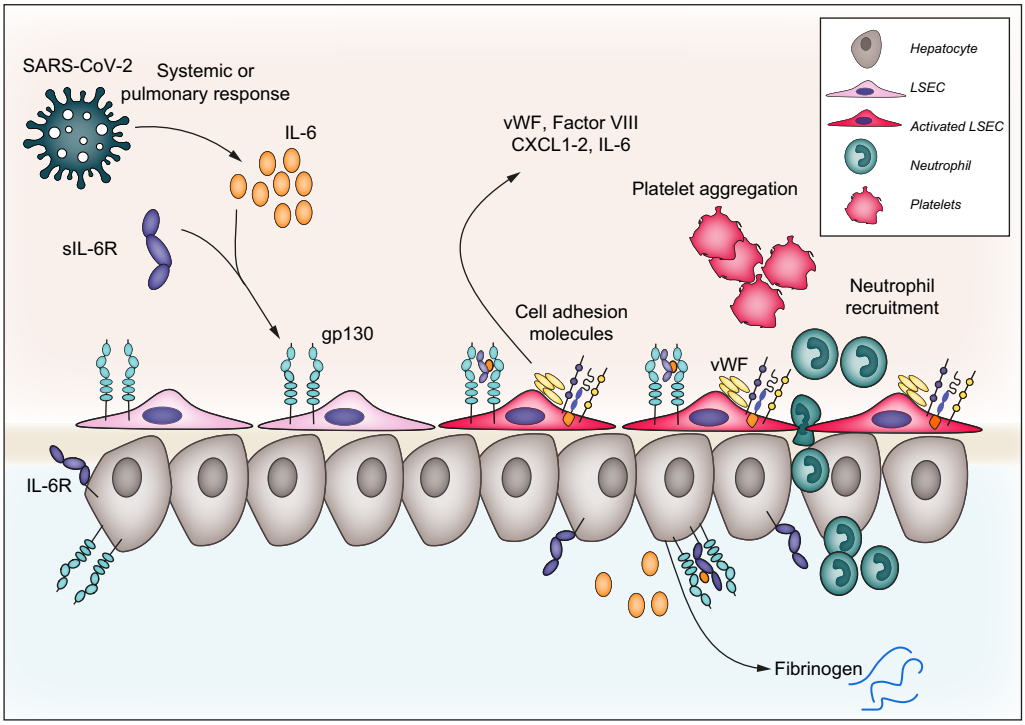

Fig. 1. Model of LSEC activation and cellular crosstalk during COVID-19 according to McConnell, Kawaguchi *et al.*[14] SARS-CoV-2 infection induces a systemic release of IL-6 which activates LSECs via a trans-signaling pathway involving the sIL-6R and gp130. Activated LSECs acquire a procoagulant and proinflammatory phenotype, secrete vWF, Factor VIII, CXCL1 and 2 and cell adhesion molecules ultimately favoring platelet aggregation and neutrophil recruitment in the liver. Moreover, LSECs produce IL-6 and cross talk with hepatocytes, which respond to IL-6 via a classical signaling pathway involving the IL-6R. Hepatocytes contribute to the systemic response to SARS-CoV2 infection by producing fibrinogen and acute phase proteins. Gp130, glycoprotein 130; IL-6, interleukin-6; IL-6R, interleukin 6 receptor; LSECs, liver sinusoidal endothelial cells; sIL-6R, soluble interleukin 6 receptor; vWF, von Willebrand factor.

infiltration. Furthermore, IL-6 is only one of several cytokines which participate in the pathogenesis of COVID-19,[20] and the role of other cytokines or liver cells (*e.g.* Kupffer cells) on LSEC activation warrants further study. Finally, while it is likely that the findings are relevant for patients with decompensated chronic liver disease who experience high liver-related mortality rates, additional studies are needed to understand COVID-19 physiopathology in this population as well as the relevance for patients without, or with mild chronic, liver disease.

Since IL-6 appears to be a major trigger of LSEC activation in the liver, it would be of interest to investigate whether IL-6 targeting compounds, especially those targeting IL-6R trans-signaling (*e.g.* olamkicept), improve liver injury.

In conclusion, although the detailed mechanisms of COVID-19-related liver injury are not yet fully elucidated, McConnell, Kawaguchi *et al.* propose a novel mechanism of liver disease pathogenesis that could be targeted to improve patient outcomes.

Financial support

T. F. B. acknowledges grant support by the European Union (ERC-2014-AdG-671231-HEPCIR), the US National Institutes of Health (U19AI12386, R01CA233794), the French National Research Agency (ANR FRM TargEnt-Covid-19), and the French Cancer Agency (ARC IHU201301187). This work has been published under the framework of the LabExANR-10-LAB-28 and Inserm Plan Cancer benefitting from a funding from the state managed by the French National Research Agency as part of the Investments for the Future program.

Conflict of interest

The authors do not declare any conflict of interest.

Authors' contributions

AS and TFB wrote the MS. AS created the figure.

Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jhep.2021.07.008.

References

Author names in bold designate shared co-first authorship

[1] Fu Y, Zhu R, Bai T, Han P, He Q, Jing M, et al. Clinical features of COVID-19-infected patients with elevated liver biochemistries: a multicenter, retrospective study. Hepatology 2020. https://doi.org/10.1002/hep.31446.

[2] Kovalic AJ, Huang G, Thuluvath PJ, Satapathy SK. Elevated liver biochemistries in hospitalized Chinese patients with severe COVID-19: systematic review and meta-analysis. Hepatology 2020. https://doi.org/10.1002/hep.31472.

[3] **Saviano A**, **Wrensch F**, Ghany MG, Baumert TF. Liver disease and COVID-19: from pathogenesis to clinical care. Hepatology 2020. https://doi.org/10.1002/hep.31684.

[4] **Moon AM**, **Webb GJ**, Aloman C, Armstrong MJ, Cargill T, Dhanasekaran R, et al. High mortality rates for SARS-CoV-2 infection in patients with pre-existing chronic liver disease and cirrhosis: preliminary results from an international registry. J Hepatol 2020;73:705–708. https://doi.org/10.1016/j.jhep.2020.05.013.

[5] **Delorey TM**, **Ziegler CGK**, **Heimberg G**, Normand R, Yang Y, Segerstolpe A, et al. COVID-19 tissue atlases reveal SARS-CoV-2 pathology and cellular targets. Nature 2021. https://doi.org/10.1038/s41586-021-03570-8.

[6] Chornenkyy Y, Mejia-Bautista M, Brucal M, Blanke T, Dittmann D, Yeldandi A, et al. Liver pathology and SARS-CoV-2 detection in formalin-fixed tissue of patients with COVID-19. Am J Clin Pathol 2021;155:802–814. https://doi.org/10.1093/ajcp/aqab009.

[7] **Ji D**, **Zhang M**, **Qin E**, Zhang L, Xu J, Wang Y, et al. Letter to the Editor: obesity, diabetes, non-alcoholic fatty liver disease and metabolic dysfunction associated fatty liver disease are proinflammatory hypercoagulable states associated with severe disease and thrombosis in Covid-19. Metabolism 2021;115:154437. https://doi.org/10.1016/j.metabol.2020.154437.

[8] Ji D, Qin E, Xu J, Zhang D, Cheng G, Wang Y, et al. Non-alcoholic fatty liver diseases in patients with COVID-19: a retrospective study. J Hepatol 2020;73:451–453. https://doi.org/10.1016/j.jhep.2020.03.044.

[9] Llitjos JF, Leclerc M, Chochois C, Monsallier JM, Ramakers M, Auvray M, et al. High incidence of venous thromboembolic events in anticoagulated severe COVID-19 patients. J Thromb Haemost 2020;18:1743–1746. https://doi.org/10.1111/jth.14869.

[10] Sonzogni A, Previtali G, Seghezzi M, Grazia Alessio M, Gianatti A, Licini L, et al. Liver histopathology in severe COVID 19 respiratory failure is suggestive of vascular alterations. Liver Int 2020. https://doi.org/10.1111/liv.14601.

[11] Ackermann M, Verleden SE, Kuehnel M, Haverich A, Welte T, Laenger F, et al. Pulmonary vascular endothelialitis, thrombosis, and angiogenesis in covid-19. N Engl J Med 2020;383:120–128. https://doi.org/10.1056/NEJMoa2015432.

[12] Lucas C, Wong P, Klein J, Castro TBR, Silva J, Sundaram M, et al. Longitudinal analyses reveal immunological misfiring in severe COVID-19. Nature 2020. https://doi.org/10.1038/s41586-020-2588-y.

[13] Investigators R-C, Gordon AC, Mouncey PR, Al-Beidh F, Rowan KM, Nichol AD, et al. Interleukin-6 receptor antagonists in critically ill patients with covid-19. N Engl J Med 2021;384:1491–1502. https://doi.org/10.1056/NEJMoa2100433.

[14] **McConnell MJ**, **Kawaguchi N**, Kondo R, Sonzogni A, Licini L, Valle C, et al. Liver injury in COVID-19 and IL-6 trans-signaling-induced endotheliopathy. J Hepatol 2021;75(3):647–658. https://doi.org/10.1016/j.jhep.2021.04.050.

[15] **Goshua G**, **Pine AB**, **Meizlish ML**, Chang CH, Zhang H, Bahel P, et al. Endotheliopathy in COVID-19-associated coagulopathy: evidence from a single-centre, cross-sectional study. Lancet Haematol 2020;7:e575–e582. https://doi.org/10.1016/S2352-3026(20)30216-7.

[16] Schmidt-Arras D, Rose-John S. IL-6 pathway in the liver: from physiopathology to therapy. J Hepatol 2016;64:1403–1415. https://doi.org/10.1016/j.jhep.2016.02.004.

[17] Li S, Jiang L, Li X, Lin F, Wang Y, Li B, et al. Clinical and pathological investigation of patients with severe COVID-19. JCI Insight 2020;5. https://doi.org/10.1172/jci.insight.138070.

[18] Zarbock A, Ley K. Neutrophil adhesion and activation under flow. Microcirculation 2009;16:31–42. https://doi.org/10.1080/10739680802350104.

[19] **Chen CX**, **Wang JJ**, **Li H**, **Yuan LT**, Gale RP, Liang Y. JAK-inhibitors for coronavirus disease-2019 (COVID-19): a meta-analysis. Leukemia 2021. https://doi.org/10.1038/s41375-021-01266-6.

[20] Ramasamy S, Subbian S. Critical determinants of cytokine storm and type I interferon response in COVID-19 pathogenesis. Clin Microbiol Rev 2021;34. https://doi.org/10.1128/CMR.00299-20.